REMARKS

Entry of this supplementary amendment and reconsideration of the above-identified application in view of the amendments above and the remarks following is respectfully requested.

The amendment to the claims made hereinabove are being made as if the amendments made in the filing of May 25, 2006 have not yet been entered, since they were Amendments made After Final Rejection and are not entered until approved by the Examiner.

The changes to the claims made in the instant Supplementary Amendment are as follows: Claims 1, 9, 18 and 27 have been further amended, and claims 52-53 have not been newly presented.

Applicants are not resubmitting the extension of time fees previously offered, nor the Declaration Under 37 CFR 1.131.

This response is prepared in light of the interview graciously granted to the Applicant on May 18, 2006. During said interview, Applicant advised the Examiner, *inter alia,* that a response was going to be filed that included both a Declaration Under 37 CFR 1.131 to remove the publication as a prior art reference, as well as a discussion of the patentability of the claim relative to the reference. Applicant also agreed to amend the claim to clarify the patentable aspects thereof.

Claims 1, 2, 6-11, 15-20, 24-29 and 33-35 are in this case. Claims 1, 2, 6-11, 15-20, 24-29 and 33-35 have been rejected. Claims 1, 9, 18 and 27 have now been amended. New claims 50-51 have now been added.

35 U.S.C. § 112, First Paragraph, Rejections

The Examiner has rejected claims 9, 15-20, 24-29 and 33-35 under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. The Examiners rejections are respectfully traversed. Claims 9, 18 and 27 have now been amended.

Specifically, the Examiner states that Applicant has not indicated where support for the limitation "**a** Ras signaling" is found. The Examiner further states that while the specification provide support for a cytoplasmic Ras mutant, it does not teach that a component of the complex signaling can be abolished.

Applicant has omitted the indefinite article "a" from claims 9, 18 and 27, thereby rendering moot Examiner's rejection in this case.

In view of the above presented amendment, Applicant believes to have overcome the 35 U.S.C. § 112, first paragraph, rejections.

35 U.S.C. § 112, Second Paragraph, Rejections

The Examiner has rejected claims 1, 2, 6-11, 15-20, 24-29 and 33-35 under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. The Examiners rejections are respectfully traversed. Claims 1, 9, 18 and 27 have now been amended.

The Examiner states that claims 1, 9, 18 and 27 recite the limitation "said Ras activity" in 1. The Examiner states that there is insufficient antecedent basis for this limitation in the claim.

The phrase "said Ras activity" in claims 1, 9, 18 and 27 has now been replaced with "said Ras signaling", thereby rendering moot Examiner's rejection in this case.

The Examiner states that claim 9 is vague and indefinite in that the metes and bounds of "indicative of an interaction between said first polypeptide and said distinct polypeptide" are unclear. Specifically, the Examiner states that the method involves expressing a library of polynucleotides each of which encodes a distinct polypeptide in cells. As multiple library members can be in each cell, it is unclear how an interaction between the first polypeptide and distinct polypeptide can be distinguished given that other interactions between distinct polypeptides and the first polypeptide are possible within the same cell.

As discussed previously, it is Applicant opinion that one of ordinary skills in the art would understand that in the embodiments of the present invention where a library of expressed (bait or prey) polypeptides is used (e.g., claim 9 and 18) each member of such a library is expressed in a specific cell of a plurality of cells. As such, only one interaction between a bait polypeptide and a prey polypeptide is possible. Cells in which such an interaction occurs survive and thus are isolated and the DNA encoding the library protein is rescued and qualified. On the other hand,

cells in which such an interaction does not occur do not survive thus greatly facilitating isolation of interaction-positive cells.

Notwithstanding the above and in-order to expedite prosecution in this case, Applicant has elected to amend claims 9, 18 and 27, to include the recitations: "expressing in each cell of a plurality of cells lacking Ras signaling"; "a second polynucleotide of a library of polynucleotides", which better define the interaction occurring between the first polypeptide and the distinct polypeptide while other interactions are not allowed since each cell comprises only one library member.

Support for the phrase "each cell" can be found in Page 11 line 14 of the instant application.

Support for the term "plurality" can be found in Page 14 line 18 of the instant application.

Support for the phrase "a second polynucleotide" can be found in Page 15 line 7 of the instant application.

The Examiner further states that claims 9, 18 and 27 are vague and indefinite in that the metes and bounds of "identifying said Ras signaling" are unclear. The Examiner points out that the cell lacks Ras signaling and hence it would not be expected that all Ras signaling is abolished in the cell. Therefore, as Ras signaling as a whole is not abolished, it is not clear how Ras signaling would only be functional under inductive conditions but not under non-inductive conditions.

In order to expedite prosecution in this case Applicant has elected to omit the indefinite article "a" from "a Ras signaling" and add the phrase "Restoration of said Ras signaling" to better define the claimed invention.

Support for the term "restoration" can be found in Page 23 line 16 of the instant application.

In view of the above presented arguments and claims amendments Applicant believes to have overcome the Examiner's rejections under 35 U.S.C. § 112, second paragraph.

35 U.S.C. § 102(a) Rejections

The Examiner has rejected claims 1, 2, 6-8, 27-29 and 33-35 under 35 U.S.C. § 102(a) as being anticipated by Takemaru and Moon, The Journal of Cell Biology

149(2), April 17, 2000. The Examiner's rejections are respectfully traversed. Claims 1, 9, 18 and 27 have now been amended.

The Examiner points out that Takemaru and Moon teach a method of identifying interactions between polypeptides comprising use of cdc25-2 yeast strain. The cells were transfected with a first polynucleotide under control of an inducible promoter encoding a polypeptide capable of interacting with a plasmalemma. The construct is library cDNA fused to v-Src myristoylation sequences and under control of the galactose inducible promoter. The cells were transfected with a second polynucleotide comprising a fusion of a second polynucleotide and a Ras cytoplasmic mutant. Cells were grown under inductive conditions, minimal galactose. The Examiner points out that the difference between the two points indicates an interaction between the first and second polypeptide.

The ability to identify protein interactions of membrane proteins is limited by prior art approaches. This may be explained by the fact that expression of these proteins in the yeast nucleus renders these proteins non-functional due to improper folding. The RRS system invented by the present inventor and practiced by Takamaru and Moon is based on the translocation of a cytoplasmic Ras to the plasma membrane via protein-protein interaction. Ras membrane recruitment results in activation of a viability pathway in yeast. However, the RRS system is limited in its ability to use membrane proteins as bait. This is due to the fact that fusion of a "bait" membrane protein to Ras will result in its membrane translocation independent of protein-protein interactions. Hence, the RRS system and specifically, the system described by Takamaru and Moon can only address interactions of soluble proteins and not membrane anchored proteins. Indeed, in the system described by Takamaru and Moon the known ("bait") protein is beta-catenin which is a soluble cell signaling protein. It should be stressed that should Takamaru and Moon have used a membrane anchored protein as bait, Ras activity would have been restored independently of protein-protein interactions. Thus, the RRS system shown by Takamaru and Moon addresses an entirely different set of proteins than the claimed invention, essentially cytoplasmic proteins.

In sharp contrast to the RRS system (practiced by Takamaru and Moon) and in order to overcome its limitations, the present inventors devised a novel

revolutionary approach which is specifically designed for the use of membrane proteins (polypeptides capable of interacting with the plasmalemma of the cell) as bait (known protein). In this system the known membrane protein is not fused to Ras to prevent Ras activation in the absence of protein-protein interactions. This approach is specifically designed for membrane receptors, ion channels and transporters which span the membrane even several times. Expression of these proteins in their natural environment preserves their unique three dimensional binding surfaces and allows the identification of true physiological interactions.

Indeed using the claimed method, the present inventors were able to identify a number of novel interactions with the membrane-coupled protein Chp, which were unknown at the time of filing of the present invention.

In order to better define the claimed invention over the prior art, Applicant has elected to amend claims 1, 9, 18 and 27 to include the limitation that the first polypeptide capable of interacting with the plasmalemma of the cell is the known protein (bait), emphasizing that the claimed invention is directed at the identification of membrane protein interactions.

Support for this claim amendment can be found in page 22 line 8 of the instant application.

Notwithstanding the above, Applicant wishes to draw Examiner's attention to the fact that Takamaru and Moon did not use a double inducible promoter system, resulting in a high level of false-positive interactions. Thus, Takemaru and Moon do not describe or suggest using double inducible promoter system (see new claims 50-1 and amended claims 18, 27) which enables the distinction between cells exhibiting Ras activity which results from expression and thus interaction with the prey polypeptide and a Ras activity which results from interaction-independent mobilization of Ras to the plasmalemma (i.e., false positive).

The following exemplifies the significance of using double inducible promoters. Empiric results suggest that about 5 % of the cDNAs fused to defective Ras will result in translocation of Ras to the plasma membrane. This is due to the fact that the cDNA encodes either a membrane protein or a protein that associates with the membrane or other membrane protein. Therefore, the fusion to defective

Ras will result in Ras membrane translocation independent of protein-protein interaction.

Following a library screen, the clones encoding such cDNA (5% of the library) will be isolated and their growth test will always be galactose dependent. Following a library screen of a million transformants, 50,000 (!) clones will be isolated due to this false positive event. The analysis of this enormous number of clones is technically laborious and time consuming.

Applicant has now elected to amend claims 18 and 27 to using such a double-promoter selection for better distinguishing the present invention from the prior art.

Thus, the presently claimed invention addresses this shortcoming, by incorporating a second inducible promoter that will express the bait protein as well under different selection (a second inducible promoter).

Support for this claim amendment can be found in Page 27, lines 3-5 of the instant application.

Thus, the present invention addresses interactions between a completely different set of proteins (membrane proteins) than that taught by the prior art (intracellular proteins) while obtaining a significantly lower level of false interactions due to the use of a double promoter system.

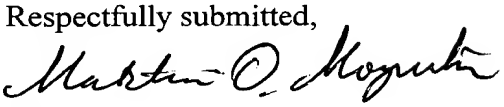
While strongly traversing the above reference, Applicant, in order to simplify the issues, attaches herewith a Declaration under 37 C.F.R. §1.131 by Applicant Ami Aronheim in which he shows a reduction to practice of the claimed invention prior to the effective publication date of Takemaru and Moon, which is the April 17, 2000. Briefly (see page 4 of the correspondence with the Editor of Nature Biotechnology) described is a novel approach to study protein-protein interactions for membrane proteins. The bait (known) protein is expressed in its natural environment, the membrane, while the prey (second polypeptide) is fused to the cytoplasmic mutant Ras. Protein interaction results in Ras membrane recruitment and activation of viability pathway. The dual inducible expression system and its significance are also described.

In view of the above declaration, the reference to Takemaru and Moon is not prior art reference relative to the instant application, and the sole rejection of the

claims over Takemaru and Moon is no longer valid and should be withdrawn.

In view of the above amendments and remarks it is respectfully submitted that claims 1-2, 6-11, 15-20, 24-29 and 50-51 are now in condition for allowance. Entry of the foregoing amendment and prompt notice of allowance is respectfully and earnestly solicited.

Respectfully submitted,



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